Evaluation of the TB Ag MPT64 Rapid test for the identification of *Mycobacterium tuberculosis* complex

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Rapid identification of *Mycobacterium tuberculosis* complex in cultured samples is important for starting appropriate treatment. We evaluated the performance of the TB Ag MPT64 Rapid test directly from 131 BACTEC™ MGIT™ 960 culture-positive samples: 113 were identified as *M. tuberculosis* complex and 18 as non-tuberculous mycobacteria. The sensitivity and specificity of the TB Ag MPT64 Rapid test were respectively 96.5% and 100% compared to the polymerase chain reaction. The overall concordance of the TB Ag MPT64 Rapid test was 96.9%. The TB Ag MPT64 Rapid test is easy, sensitive, and does not require a high level of skill or specific equipment.

**KEY WORDS:** tuberculosis; nitrate reductase; MGIT 960; drug resistance

IN RECENT YEARS, culture capacity for the diagnosis of tuberculosis (TB) has improved, particularly in low-income countries, due in part to the new policy recommendations of the World Health Organization on the use of liquid-based medium culture issued in 2007.¹ For any positive liquid culture obtained, laboratories need to use a rapid and affordable method for the identification of the mycobacterial species to differentiate *Mycobacterium tuberculosis* complex from non-tuberculous mycobacteria (NTM). Identification of mycobacteria is usually performed by culture and biochemical tests that take several weeks to produce results due to the slow growth of mycobacteria. The procedures are laborious and time-consuming, and can fail to correctly identify the mycobacteria.²,³

The predominant secreted protein released during growth of *M. tuberculosis* in culture medium is the MPT64 antigen.⁴ A new simple immunochromatographic lateral flow assay (strip test in cassette) has recently been developed. The TB Ag MPT64 Rapid test (SD Bioline, Standard Diagnostics, Suwon, Korea) detects the protein MPT64 in liquid or solid cultures and can be easily used for the rapid identification of *M. tuberculosis* complex without any technical complexity.

In this study, we evaluated the TB Ag MPT64 rapid test for the identification of *M. tuberculosis* directly from positive BACTEC™ MGIT™ 960 cultures (BD Diagnostic Instrument Systems, Sparks, MD, USA).

**MATERIAL AND METHODS**
**Samples**
One hundred and thirty-one respiratory samples were decontaminated according to standard procedures by the Petroff method.⁵ Five hundred microlitres were inoculated into BACTEC MGIT 960 according to the manufacturer’s instructions.

**Identification of mycobacteria**
BACTEC MGIT 960 cultures that gave a positive signal were checked for acid-fast bacilli (AFB) by Ziehl-Neelsen staining. Only AFB-positive samples were included in this study. If the stain was negative for AFB, the sample was considered contaminated and eliminated from further study. All samples were tested by the TB Ag MPT64 Rapid test within 72 h of giving a positive signal. Contamination of the sample was checked by inoculating some drops in a blood agar medium. After 48 h, the blood agar plate was checked for contamination.

The gold standard method for the identification of mycobacteria was the sequencing of the 16S rRNA⁶ and polymerase chain reaction (PCR) for the specific identification of *M. tuberculosis* complex.⁶,⁷

**TB Ag MPT64 Rapid test**
A volume of 100 μl of liquid culture from a positive MGIT 960 vial was applied directly to the TB Ag MPT64 Rapid test. Results were interpreted after
The presence of two colour bands (control and test bands) was considered as positive. A colour band of any intensity was interpreted as a positive result. The presence of the control band alone indicated a negative result. If the control band was not visible after 15 min, the test was considered invalid and the sample was retested.

RESULTS

The results of the 131 BACTEC MGIT 960 culture-positive samples tested by the TB Ag MPT64 Rapid test and by PCR are shown in the Table. Of the 131 positive cultures, 113 were identified as M. tuberculosis complex by PCR and 18 as NTM. We further identified the 18 NTM by sequencing the 16S rRNA gene and found that M. intracellulare was the NTM most frequently isolated (10/18); 2 M. chelonae, 1 M. asiaticum, 1 M. brasiliensis, 1 M. flavescens, 1 M. parascrofulaceum, 1 M. terrae and 1 atypical mixture were also identified.

The TB Ag MPT64 Rapid test failed to detect four M. tuberculosis isolates. The BACTEC MGIT 960 gave a positive signal after 2 days for two samples, and the samples were highly contaminated (blood agar gave a positive signal after 2 days for two samples, and isolates. The BACTEC MGIT 960 mixture were also identified.

The TB Ag MPT64 Rapid test was respectively 96.5% and 100%.

DISCUSSION

This study evaluated the performance of the TB Ag MPT64 Rapid test for culture identification of M. tuberculosis. The TB Ag MPT64 Rapid test is easy, sensitive and does not require a high level of skill or dedicated equipment for the identification of mycobacteria in liquid culture medium. All positive cultures in liquid media can be used directly for the TB Ag MPT64 Rapid test without any sample preparation or dilution. Faint bands observed in the cassette are considered as positive and correlate well with PCR results. Discordant results have been observed only in highly contaminated samples or with a low expression of the antigen in the medium.

Hirano et al. showed that Capilia TB-negative isolates had mutations within the mpt64 gene, causing an incomplete protein production. We did not find this problem in our study. Park et al. showed that the TB Ag MPT64 Rapid test had an excellent sensitivity (99%) and specificity (100%) and an appropriate detection limit of 10^5 colony forming units/ml. The Capilia test, a similar test, is not available on the market but only through FIND (Foundation for Innovative New Diagnostics, Geneva, Switzerland) and for a restricted number of countries, particularly the high TB burden countries. Middle- and high-income countries also urgently need identification tests that can be combined with liquid culture systems available on the market.

Becton Dickinson has recently also released a new immunochromatographic test (TBc ID) based on the same simple technology. This test is currently under evaluation in our laboratory.

The cost of the TB Ag MPT64 Rapid test in Belgium (market price) is €3 compared to the cost of PCR for M. tuberculosis complex, which was estimated to be around €12 per test. In South Africa, the cost of the same test was approximately one third of the price of the current molecular probe method. In Thailand, the price of the Capilia test (based on a discounted price negotiated through FIND) was US$2.67. The time to perform the TB Ag MPT64 Rapid test is only 15 min compared to PCR, which requires at least 3 h.

Rapid identification tests require appropriate bio-safety measures for the manipulation of M. tuberculosis cultures within a containment laboratory, and tests should be performed within a well functioning biosafety cabinet. We recommend using the TB Ag MPT64 Rapid test only in non-contaminated samples to avoid false-negative results. In this study, two highly contaminated M. tuberculosis samples could not be identified by the TB Ag MPT64 Rapid test. A negative result does not rule out infection by M. tuberculosis or the co-existence of M. tuberculosis and NTM. There is hope that the rapid speciation test will supplement other laborious identification tests that are beyond the capacity of most laboratories other than reference laboratories.

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References


L’identification rapide du complexe *Mycobacterium tuberculosis* dans les échantillons mis en culture est d’importance extrême si l’on vise à mettre en route le traitement approprié chez les patients infectés. Nous avons évalué les performances du test TB Ag MPT Rapid directement à partir de 131 échantillons positifs à la culture au BACTEC™ MGIT™ 960. Cent treize ont été identifiés comme appartenant au complexe *M. tuberculosis* et 18 comme mycobactéries non-tuberculeuses (NTM). La sensibilité et la spécificité du test TB Ag MPT64 Rapid ont été respectivement de 96,5% et 100% par comparaison avec la PCR. La concordance globale du test TB Ag MPT64 Rapid a été de 96,9%. Le test TB Ag MPT64 Rapid est aisé, sensible, et n’exige ni un niveau élevé de compétence, ni un équipement spécifique.

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**RÉSUMÉ**

La détection rapide du complexe *Mycobacterium tuberculosis* en las muestras de cultivo es de máxima importancia, con el objeto de iniciar un tratamiento adecuado a los pacientes infectados. Se evaluó la eficacia de la prueba de diagnóstico rápido de la tuberculosis con el antígeno MPT64, directamente a partir de 131 muestras de cultivo positivas en el sistema BACTEC™ MGIT™ 960. Se detectaron 113 cepas del complejo *M. tuberculosis* y 18 cepas de micobacterias atípicas. La sensibilidad de esta prueba rápida fue 96,5% y su especificidad fue 100%, en comparación con la prueba de reacción en cadena de la polimerasa. La concordancia global de la prueba rápida fue 96,9%. La prueba con el antígeno MPT64 constituye un método sencillo y sensible y no exige un alto grado de competencia ni equipos específicos.